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TITLE: Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

PRINCIPAL INVESTIGATOR: James R. Hebert, Sc.D.

CONTRACTING ORGANIZATION: University of South Carolina
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13. ABSTRACT ***(Maximum 200 Words)***

This combined case-comparison study and randomized controlled trial (RCT) of 90 women is based on our prior epidemiologic work (1-6) indicating that vegetables in the Brassica genus (e.g., broccoli, cauliflower, brussel sprouts) can modify estrogen metabolism by causing 17β- Estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16α-hydroxyestrone (16HE) thus producing a cascade of effects protective against breast cancer (2). Our plan is to enroll 45 postmenopausal women with breast cancer and 45 age-matched disease-free women and to compare them on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1□; and 2) levels of relevant estrogens, E2, 2HE, and 16HE.

The RCT will examine the effect of an intensive Brassica-rich diet intervention on AhR activation, its protein products, and estrogen metabolites in these women. This study is beginning its second full year of funded activity. All protocols for the collection of data are finalized and we have begun to recruit participants. The baseline data are being collected for case-comparison study and the first of four intervention cycles for the RCT began in 2002. Specific accomplishments are described in the following narrative, in parallel with the original Statement of Work.

1. Fowke JH, Longcope C, Hebert JR. Macronutrient intake and estrogen metabolism in healthy postmenopausal women. Breast Cancer Res Treat 2001; 65:1-10.
2. Fowke JH, Longcope C, Hebert JR. Brassica vegetable consumption shifts estrogen metabolism in healthy postmenopausal women. Cancer Epidemiol Biomark Prev 2000; 9:773-779.
3. Hebert JR, Hurley TG, Ma Y. The effect of dietary exposures on recurrence and mortality in early stage breast cancer. Breast Cancer Res Treat 1998; 51:17-28.
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5. Hebert JR, Wynder EL. Dietary fat and the risk of breast cancer. N Engl J Med 1987; 317:165-166.
6. Hebert JR, Toporoff E. Dietary exposures and other factors of possible prognostic significance in relation to tumor size and nodal involvement in early-stage breast cancer. Int J Epidemiol 1989; 18:518-526.

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Army Award DAMD17-99-1-9279

Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

Annual Report: Year 3

This study is beginning its third full year of funded activity. All protocols for the collection of data are finalized and we have begun to recruit participants. The first of four intervention cycles began in 2002. Specific accomplishments are described in the following narrative, in parallel with the original Statement of Work. We have ~60 women enrolled in the study so far (October 2002).

Introduction

Work by our group and others provide the scientific basis of this study (1-11). Cross-national studies of breast cancer rates and studies of migrants indicate that environmental factors are responsible for large population-level differences in breast cancer rates and rates of change over time. In a study of 46 countries, we found that over 90% of breast cancer mortality could be accounted for mainly by dietary factors (12). On a per-calorie basis, the strongest effect in the data was the protective effect of cabbage. There is some evidence that vegetables in the *Brassica* genus, like cabbage and broccoli, modify estrogen metabolism by causing 17β- Estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16α-hydroxyestrone (16HE). Relative to 2HE, 16HE appears more likely to cause cancer and breast cancer patients have a lower ratio of these metabolites than do disease-free controls. It has further been shown that the P450 enzyme CYP1B1 is present in tumor but not normal breast tissue. The indole glucosinolates (IGSL), which are contained in high concentrations in Brassica vegetables, induce a number of protein products that can shift E2 metabolism away from 16HE and towards 2HE. AhR activation also induces immune system factors such as interleukin-1β (IL-1β) and other proteins, such as plasminogen activator inhibitor-2 (PAI-2), a protease inhibitor that has been associated with inhibition of tumor invasiveness (metastasis).

Specific Aims

The two objectives of this proposal are to evaluate the products of AhR activation against the risk of breast cancer, and to investigate the ability of *Brassica* vegetables to reduce breast cancer risk. Women will be recruited from among those who have undergone a diagnostic biopsy at SCCC following a suspicious mammogram. The plan is to enroll 45 postmenopausal women who have had breast cancer and 45 age-matched women found to be disease free. The first study, conducted at the time the women enter the study, will compare the 45 breast cancer patients and the 45 high-risk healthy women on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1β; and 2) levels of relevant estrogens, E2, 2HE, and 16HE. The second study will examine the effect of an intensive Brassica-rich diet intervention on AhR activation, its protein products, and estrogen metabolites in these 90 women. Measurement of all study parameters will be made at times corresponding to

the baseline period and post-intervention. Blood and fasting morning urine samples will be collected for measurement of the estrogens, and levels of PAI-2 and IL-1β. Adipose tissue for assay of CYP1B1 will be collected from routine open biopsy at the time of recruitment and from a fine needle biopsy of the contralateral breast at follow-up. Diet will be assessed by use of validated diet assessment instruments. Compliance also will be assessed by levels of isothiocyanates and dithiocarbamates in urines. Statistical analyses of the data will consist of t-tests and analysis of variance of mean levels of the parameters specified in the three groups at baseline. T-tests of change and regression analyses (e.g., repeat measures ANOVA) will focus both change and relative change in the intervention trial. Post hoc analyses will examine the effect of the indole carbinols by fitting the data as continuous, which takes into account varying levels of compliance.

Distinctive subject terms

- Brassica vegetables- vegetables belonging to the Brassica genus including cabbage, broccoli, cauliflower, spinach, collards, and Brussels sprouts
- Brassica diet- consuming an intensive Brassica-rich diet
- Indole glucosinates (IGSL)- Dietary indoles are contained in Brassica vegetables and converted in the body to aryl hydrocarbon receptor (AhR) agonists that bind to AhR and induce CYP1 enzymes.
- Aryl hydrocarbon receptor (AhR)- has a role in inducing protein products that can shift E2 metabolism away from 16HE and towards 2HE. It has a role in inducing immune system factors (e.g., interleukin-1β and other proteins (e.g., plasminogen activator inhibitor-2)
- Hydroxyestrone- two forms of this hormone are created using the 17α-Estradiol precursor (e2) including 2-Hydroxyestrone (2HE, less toxic form) and 16α-Hydroxyestrone(16HE, more toxic form)
- Cytochrome P1B1 (CYP1B1)- a phase I enzyme present in tumor but not in breast tissue

The primary hypotheses are:

1. Examine if there are differences in AhR and its protein products, including CYP1B1, PAI-2, and IL-1□ and estrogen metabolites at baseline in two subsets of women who have undergone diagnostic open breast biopsy at SCCC;
2. If intensive Brassica vegetable intake can alter levels of these products and estrogen metabolites through intensive dietary intervention on Brassica vegetable intake; and
3. If there is a relationship between CYP1B1 and estrogen metabolites, both cross-sectionally and longitudinally.

Work Accomplished

We have modified all questionnaires being used and obtained access to the Palmetto Health Association's (PHA) tumor registry database. We have begun active recruitment of potential study participants. Potential women have been identified from the PHA's Tumor Registry and from the Breast Care Centers at both Richland and Baptist hospitals. Intervention cycles began in April.

Task 1: Run-in Phase, Months 1-12:

a. Inventory and finalize all assessment instruments and data collection protocols.

Assessment instruments have been inventoried and are available for use. Final versions of all assessment instruments have been produced, as stipulated in the protocol. Copies of these instruments are included in the appendix.

Below is a list of instruments being utilized.

Baseline questionnaire Measures include: Background and Demographic Data: age; sex; marital status; education; number of children; number and dates of pregnancies; breast feeding history: (months for each child); and menopausal status (including surgical menopause). Personal Health History: present medical/psychiatric history and treatment (including history of exposure to estrogens, oral contraceptives, unusual menstrual problems). Family Health History: history of breast cancer; history of other cancers. General Self Care: sleep; exercise frequency; and smoking status.

Besides data collected on the baseline instrument we will also administer these other questionnaires:

- Marlowe-Crowe Social Desirability (MCSD) scale (Personal Reaction Inventory)
- Social Approval Scale
- Multiple 24-Hour Recall Phone Interviews [note that we have changed to this method as it appears to ease participant burden and is associated with lower overall measurement error (13).]
- Vegetable and Fruit Questionnaire [the paper validating this was published recently (14)
- Monitoring questionnaire
- Intervention Course Book, which includes intervention descriptions, food preparation methods, a cook book, telephone numbers of study personnel, and a brief description of the purposes of the study

New data collection protocols have been developed to fully utilize all resources under development at USC. As part of standard recruitment procedures, we mail an introductory letter and consent form to potential participants. We follow-up this letter with a telephone calls, and answer any questions regarding the study. As part of recruitment, a meeting is scheduled at the study center located within the South Carolina Cancer Center (SCCC). The SCCC facility includes an interview room, sample processing lab, and calibrated scales and measurement instruments. At the meeting, participants have the opportunity to ask additional questions regarding the consent form. After obtaining consent, we obtain a urine sample, blood sample, buccal cells, body size measurements, and participants complete the baseline questionnaire. Follow-up measurements are collected using a similar mechanism. Additionally, near the end of the intervention a clinic appointment is scheduled for collection of breast biopsy material, a fasting urine sample, body size measurements, and participants complete a vegetable and food survey (see above).

b. Review baseline questionnaires for completeness and for content validity.

All instrument materials have been thoroughly reviewed and validated.

c. Revise baseline questionnaire to assess demographic, health history, and family health history, as necessary.

The Baseline Questionnaire has been expanded to include a more complete description of each participant's health history and demographic status. This expansion followed the move to USC, and the greater population diversity in SC as compared to Massachusetts. The questionnaire has been pilot tested, and appears to be sufficiently clear and complete.

d. Hire and train the Research Assistant.

Several personnel have been hired in order to complete this, and other, research projects. Dr. James Hebert will remain the Principal Investigator for the project and Dr. Jay Fowke has assumed the role of consultant. Dr. Stephanie Muga remains the as Co-Investigator for the study. Wendy McKenzie is now acting as Project Coordinator. Mary Modayil, a USC doctoral student in the Department of Epidemiology will be largely responsible for the day-to-day operations of the project. Thomas Hurley functions as a full-time data manager. His primary responsibility focuses on developing the tracking databases necessary for ensuring complete recruitment and data collection. Additionally, he is responsible for questionnaire maintenance, questionnaire development, and data entry. Yasmin Khan, Krystal Hanrahan, and Catishia Mosely are Masters students in the Department of Epidemiology. Their primary responsibility will be to assist Dr. James Hebert in contacting potentially eligible participants, mailings, and data management.

e. Develop the study data management systems, using a combination of Lotus Notes, Microsoft Excel, and EpiInfo.

As mentioned in the previous report, we have developed an improved data management system using optical scanning technology and the Teleform software package. Lotus Notes was not used in this study as we have moved to more universally recognized solutions. All questionnaires are now optically scanned, thus avoiding operator error associated with keypunching data, and greatly speeding the data entry process. Optically scanned data are directly transferred to a SAS dataset for analysis, thus eliminating most of the need for EpiInfo.

f. Develop the tracking database in Microsoft Access and Microsoft Excel based on our experience with other intervention studies in the Department of Epidemiology and Biostatistics.

We are in the process of refining an extensive database system, which links directly with the clinical hospital patient bases and other ongoing cancer studies. This data management system is able to rapidly identify potentially eligible women receiving care at one of the

cancer centers. This information is converted to the study-specific tracking system, used for maintaining records of recruitment, participant status, and data collection.

g. Train staff in all data-related and clinic-based procedures.

We have trained staff to conduct all data-related procedures. Dr. Hebert, Mr. Hurley, and Ms. Modayil are responsible for the overall data management and statistical analysis. Mr. Hurley, the data manager, has received formal training in the Teleform software package and extensive experience using the SAS software package. The graduate research assistants have been trained in the application of Teleform and they are developing the skills necessary to perform many routine SAS data management operations. They also have been trained to collect body size measurements using standard and systematic protocols, as well as in urine collection, sample preparation, and storage protocols. The biopsy collection protocol will be conducted by one of the members of the Radiology Department with the PHA hospital network.

h. Develop and finalize all laboratory procedures to be used in the trial.

The majority of laboratory procedures will be conducted by Dr. Stephanie Muga at USC. With the exception of the CYP1B1 assay, all necessary laboratory protocols are commercially available as kits. Members of Dr. Muga's lab have extensive experience in forming radioimmunoassays and enzyme immunoassays as required through use of these kits.

i. Finalize all biological sample collection and storage procedures to be used in the study.

All biological sample collection and storage procedures for urine and blood are finalized. The biopsy collection protocol has been developed in order to maximize volume of epithelial cells from breast tissue, due to new published findings suggesting better methods to detect CYP1B1 in breast tissue. The assay protocol is almost finalized with the help of Dr. Muga's lab with the goal of increasing sensitivity of the antibody to the CYP1B1.

j. Establish recruitment procedures for women entering the study, including pre-screen for certain criteria such as menopausal status.

Recruitment procedures have been established and we are beginning to recruit women. We are mailing an initial recruitment card to height awareness of the study. This is being followed by an information letter relating more study details. Both of these recruitment methods also mention the upcoming phone interview during which we are collecting pre-screening information on personal characteristics, diet, medication use, and health history. We will identify women seeking a screening mammogram at one of the clinical centers within the PHA. We have developed the data management system such that we will be able to identify women who receive a negative screening (healthy) and women who eventually are diagnosed with breast cancer.

k. Finalize the intervention protocol.

We have finalized the intervention protocol, based on our experiences with past dietary interventions. An intervention syllabus has been generated, listing specific content and topics for each class. Our dieticians, Brook Harmon and Lori Myers will lead weekly group discussions on incorporating Brassica vegetables into a daily diet, menu planning, and preparing quick healthy meals. Intervention materials have been generated, including a course booklet, 3-day diet diaries, a brief vegetable questionnaire, a brief monitoring questionnaire designed to measure adverse reactions or changes in health-related behaviors, and a recipe book. Dietary goals have been set. Rapid conversion of self-reported compliance levels will allow participants to monitor compliance relative to peers. We have identified several dieticians in Columbia who are sufficiently skilled to lead the intervention, and we are confident in our ability to hire such an intervention leader at the appropriate time.

Task 2: Recruitment, Months 12-24:

a. Identify women who could be eligible for the study from among those visiting the Breast Clinic at Richland Memorial Hospital for the purpose of an open biopsy as a part of a diagnostic work up following a suspicious mammogram. We have also identified former breast cancer cases from the PHA Tumor Registry Database who may be eligible to take part in this study. We will primarily sample from this registry for the first cycles of the intervention. We have mailed recruitment information to these women and have begun phone interviews.
b. We have put into place procedures for recruitment through the PHA clinical services. We will be able to identify women receiving breast biopsy procedures and who could be eligible for the study among those visiting the PHA participating hospitals. Recruitment has begun (January to October 2002).
c. Among those who say they are willing to participate, determine eligibility using the 18 criteria listed in section 4.1 of the proposal. We have developed a simple eligibility screening form suitable for use in the large-scale screening of potential participants during a telephone interview.
d. Abstract medical records for relevant health history and pathology data. The PHA Tumor Registry contains information on pathology and the history of the first course of treatment for women with a previous diagnosis of the disease. For women currently visiting the Breast Clinic at Richland Memorial Hospital or Baptist Hospital, we are able to link their medical records with eligibility criteria in order to enroll them into this study.
e. Randomize to either intervention or control. Inform woman of this. When woman attend their 1st clinic visit, they are assigned to the intervention or non-intervention group.
f. Enroll the consecutive eligible women who have histologically confirmed stage I or II cancer of the breast. We have completed data collection on 10 women who have had breast cancer.
g. Enroll consecutive eligible women who are disease free and meet all eligibility requirements of the study and are matched to the cases on age (±5 years). We have completed data collection on 38 women who are disease free and meet all eligibility requirements of the study.
h. Schedule the first clinic appointment for the purposes of collecting all of the blood and urine specimens and taking the anthropometric measurements.

i. Ensure that the open biopsy material is processed and sent to Dr. Muga's laboratory. Biopsy material is kept frozen and in storage.
j. Collect data on lifestyle, demographic, and health (family and personal history) plus psychosocial factors as outlined in 4.4.3. We use the baseline survey for this.
k. The dietician contacts each participant randomized to the intervention and schedules the group sessions. If the participant cannot attend all classes, the dietician conducts individual sessions with the participant on the telephone.

Task 3: Intervention / Passive Follow Up in the Controls, Months 14-28 (all items subsumed here are on-going):

Ensure that the intervention is delivered according to the protocol.

a. Through collaboration with a local cardiac rehabilitation center, we have access to an appropriate conference room and adjoining teaching kitchen.
b. Encourage women randomized to the intervention to attend all of the sessions. The dietician contact women to encourage them to attend. The women are provided adequate vegetables for the intervention during the group sessions.
c. Stay in contact with the control group to assure compliance with the follow-up measures.
d. Schedule the follow-up visit at the Breast Clinic for the blood, urine, and anthropometric data collection.
e. Schedule the visit for the needle biopsy at the Breast Clinic.
f. Assure that all self-assessments are completed at follow up.

Task 4: Data Entry, Verification and Interim Analyses, Months 12-28 (all items subsumed here are on-going):

a. Assure that all data are immediately read into the tracking and analytic databases.
b. Flag all outlier and illogical responses.
c. Verify all outlier and illogical responses, re-contacting participants, if necessary.
d. Conduct simple descriptive analyses (e.g., cross-tabulations and univariate statistics).

Task 5: Final Data Analyses, months 28-36:

a. Perform all exploratory analyses to test for adherence to model assumptions.
b. Perform all necessary data manipulations (e.g., log transforming all non-normal and heteroschedastic data).
c. Test study hypotheses.
d. Conduct post-hoc analyses of study data.
e. Prepare manuscripts.
f. Archive datasets for future analyses and future patient follow-up.
g. Plan for future studies.

Key Research Accomplishments are all subsumed under the Task List, as noted above.

Reportable Outcomes, in addition to those things noted above, include two papers of relevance to this study including one on using isothiocyanate excretion as a biological marker of *Brassica* vegetable consumption (14) and the other on nutrient intake and estrogen metabolism in healthy postmenopausal women (15). Copies of these are included in the appendix. We also have produced a large number of measurement instruments that are included in the Appendix as well. Preliminary data analysis was presented at the Era of Hope Conference in September 2002. (see appendix)

Conclusion: After experiencing delays with study start up due to issues around Human Use, this study is now on track in terms of research deliverables.

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15. Fowke JH, Longcope C, Hebert JR. Macronutrient intake and estrogen metabolism in healthy postmenopausal women. Breast Cancer Res Treat 2001; 65:1-10.

Appendices

Appendix 1: Curriculum Vitae	Wendy McKenzie- Biosketch
Appendix 2: Assessment Instruments	Baseline Questionnaire * Vegetable and Fruit Questionnaire * Side-Effects and Reactions Form *
Appendix 3: Recruitment and Consent	Recruitment Card * Letter of Introduction * Consent Form Phone Script * Screening Survey
Appendix 4: Collection & Processing	Urine* Blood* Body Size Measurements *
Appendix 5: Intervention Materials	Draft Syllabus* Food Lists and Dietary Goals* 24-HR Recall Script *
Appendix 6: Preliminary Data Analysis	Era of Hope Poster Presentation

*Indicates these are unchanged from those filed in the previous report.

Appendix 1
Curriculum Vitae

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Wendy Bush McKenzie	Program Manager

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Francis Marion College, Florence, SC University of South Carolina, Columbia, SC	B.S.	1990 2000-2002	Sociology Public Health

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

January 2000 – August 2002 Project Coordinator, College of Nursing, University of South Carolina
August 2002-present Program Manager, Department of Epidemiology and Biostatics, University of South Carolina

Appendix 2
Assessment Instruments

2017326450

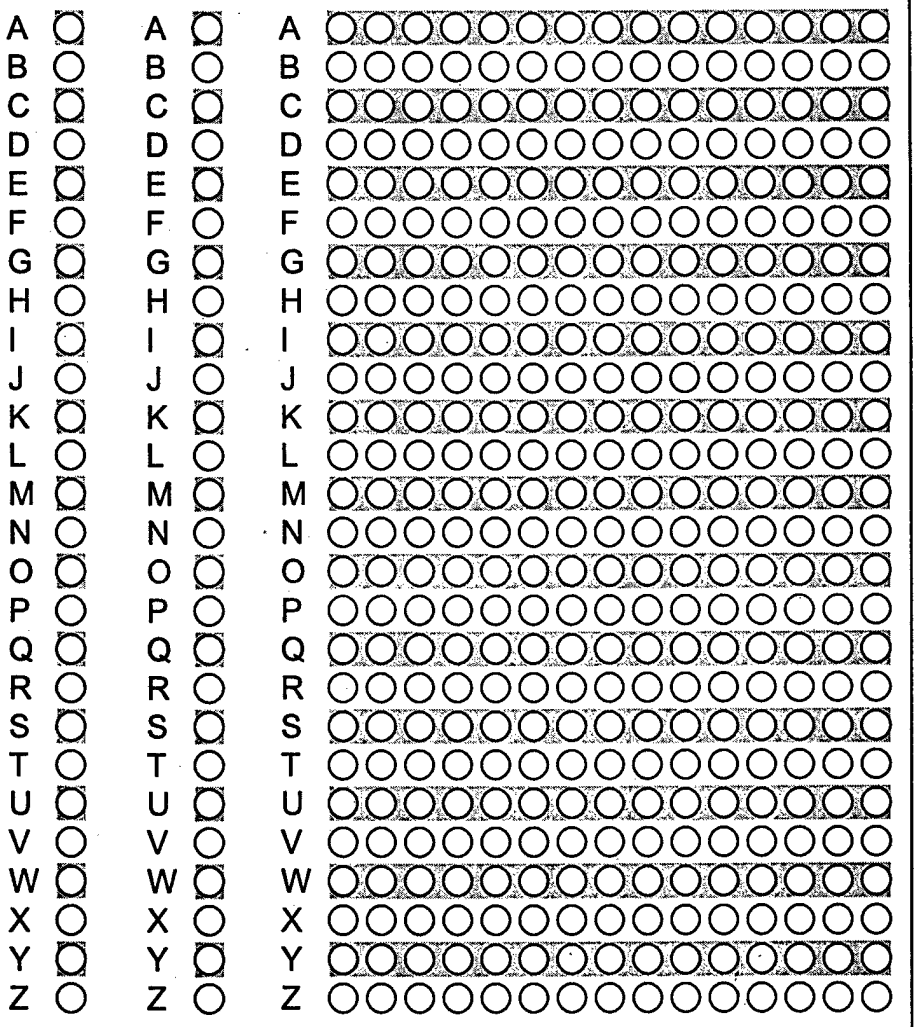
ID

BRASSICA HEALTH STUDY Baseline

Date Form Completed

Month / Day / Year

First Initial **Middle Initial** **Last name**



Contact Information:

E-mail address (Optional): _____ @ _____

First some questions about your personal characteristics.

1. **How tall are you (without shoes)?** ☐ Feet ☐☐ Inches

2. **What is your current weight?** ☐☐☐ Pounds

3. **Are you: (Specify more than one, if applicable)**

- ○ White (Non-Hispanic)
- ○ Black (Non-Hispanic)
- ○ Hispanic/White
- ○ Hispanic/Black
- ○ Asian
- ○ Other (specify):

4. **What is your current marital status? (Select only one.)**

- ○ Married
- ○ Living with a partner
- ○ Widowed
- ○ Divorced
- ○ Separated
- ○ Single, never married and not living with a partner

5. **How would you describe your religion? (Mark ONE only)**

- ○ Roman Catholic
- ○ Protestant
- ○ Jewish
- ○ Muslim
- ○ Other (Specify): _____
- ○ No Religion (go to question #7)

6. **Do you regularly (at least once monthly) attend religious services?**

O Yes
O No

7. **What is the highest year or level of school you have completed? (Select only one.)**

○ 8th grade or less

○ More than 8th grade and less than high school

○ High school completed, no college

○ High school completed, some college (Associates degree, RN, etc.)

○ College completed (BS, BA, BSN, etc.)

○ More than college completed (MA, MS, PhD, etc.)

8. **Are you presently employed? (Select only one.)**

○ Yes, employed full time

○ Yes, employed part time

○ No (go to question #13)

9. **If employed, how do you classify your usual position? (Select only one.)**

○ Skill or craft
○ Machine operator
○ Manual labor
○ Sales
○ Scientific/Technical work
○ Service work
○ Clerical or office
○ Professional, managerial or administrative

10. We would like to know about your activity at your job.
(Please fill in one circle for each item)

	Never	Seldom	Sometimes	Often	Always
a. At work, I sit...	○	○	○	○	○
b. At work, I stand...	○	○	○	○	○
c. At work, I walk...	○	○	○	○	○
d. At work, I lift heavy objects...	○	○	○	○	○
e. At work, I am tired...	○	○	○	○	○
f. At work, I sweat...	○	○	○	○	○

11. Did anyone at your workplace smoke cigarettes, cigars, or a pipe in the past week?

○ Yes

○ No (Go to question 13)

12. If YES, please indicate the type of tobacco smoke and amount of time you were exposed to tobacco smoke at the workplace in the past week.

Smoke Source	Amount of time in last week that you were in the same room or car as a coworker that smoked tobacco
Cigarette	
Cigar	
Pipe	

13. Were you exposed to cigarette, cigar, or pipe smoke anywhere outside the workplace (for example: at home, at a friend's house, or eating out) in the past week?

O Yes
O No (Go to question 15)

14. If YES, please indicate the type of tobacco smoke and amount of time you were exposed to this smoke in the past week.

Smoke Source	Amount of time in last week that you were exposed to smoke, not at work
Cigarette	
Cigar	
Pipe	

15. Do you ever drink any alcoholic beverages (i.e. beer, wine, spirits/liquor)?

O Yes
O No (Go to question 17)

16. If yes, then please indicate how much of each beverage you drink in a typical week.

Beverage		*Typical # drinks in a week*	
a) Beer	O Yes O No	☐☐	bottles or cans (12 oz.)
b) Wine	O Yes O No	☐☐	glasses (6 oz.)
c) Spirits	O Yes O No	☐☐	drinks (1.5 oz. liquor)

Next, some questions about your pregnancy history and children.

17. Have you ever been pregnant?

O Yes
O No (Go to question 22)

18. Have you ever had a pregnancy that lasted beyond the first trimester (in other words, past the first three months)?

O Yes
O No (Go to question 20)

19. Please list dates of your pregnancies that lasted beyond the first trimester, the result of the pregnancy, and the sex of the child.

Pregnancy No.	*Date Pregnancy Ended (mm/dd/year)*	*Result*	*Sex (M/F)*
1	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female
2	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female
3	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female
4	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female
5	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female

Pregnancy No.	*Date Pregnancy Ended (mm/dd/year)*	*Result*	*Sex (M/F)*
6	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female
7	☐☐ / ☐☐ / ☐☐☐☐	O Live Birth O Still Birth O Other Loss	O Male O Female

20. Have you ever had a first trimester miscarriage or abortion?

O Yes
O No (Go to question 22)

21. If so, please provide the number of first trimester miscarriages or abortions: ☐☐

22. Do you have children?

O Yes
O No (Go to question 26)

23. How many children do you have?

☐☐ Biological children

☐☐ Adopted children

☐☐ Step children

☐☐ Total number of children

24. How many children currently live with you? ☐☐ Children

25. What is the age of the youngest child living with you? ☐☐ Years Old

26. **How old were you when your periods or menstrual cycle started?**
(Please be as accurate as possible.)

☐☐ Years Old

27. **Have you ever had menstrual problems?**

O Yes
O No (Go to question 29)

28. **If yes, which of the following problems have you experienced?**
(Please indicate all that apply to you.)

☐ **Cramps**
☐ **Irregular periods**
☐ **Heavy bleeding**
☐ **Other (please describe):** _____

_____

_____

29. **Have you had a period in the last 12 months?**

O Yes (Go to question 32)
O No

30. **How old were you when your periods stopped?**
(Please be as accurate as possible.)

☐☐ Years Old

31. **Why did your periods stop?**
(Please indicate all that apply to you.)

O **They stopped naturally.**
O **They stopped as a result of a hysterectomy.**
O **They stopped as a result of another surgical procedure.**
O **They stopped as a result of a medicine or therapy**
O **They stopped due to other reasons. (please describe):** _____

_____

_____

32. Have you ever had a breast biopsy, where a doctor removed some breast tissue either surgically or with a needle?

- O Yes
- O No (Go to question 35)

33. If YES, how many surgical breast biopsies have been performed? ☐☐ Surgical biopsies

34. How many needle breast biopsies have been performed? ☐☐ Needle biopsies

---

Now, some questions about the activities you did in your home last week.

35. Did you do the light household work (dusting, washing dishes, mending/sewing)?

- O Never
- O Sometimes (only when partner or help not available)
- O Mostly (sometimes assisted by partner)
- O Always (alone or with partner)

36. Did you do the heavy household work (washing floors, windows, carry trash, etc.) ?

- O Never
- O Sometimes (only when partner or help not available)
- O Mostly (sometimes assisted by partner)
- O Always (alone or with partner)

37. For how many people did you keep house including yourself? ☐☐ Person(s)
(Fill in '0' if you answered 'never' to Questions 35 and 36)

38. How many rooms did you keep clean, including kitchen, bedroom, garage, cellar, bathroom, attic, etc.?

- O Never do housekeeping (Go to question 40)
- O 1 to 6 rooms
- O 7 to 9 rooms
- O 10 or more rooms

39. If any rooms, on how many floors in your home are these rooms? ☐☐ Floor(s)

40. Did you prepare warm meals yourself, or did your assist in preparing?

- O Never
- O Sometimes (1 to 2 times a week)
- O Mostly (3 to 5 times a week)
- O Always (more than 5 times a week)

41. How many flights of stairs did you walk on a typical day (one flight of stairs is 10 steps)?

- O I never walk stairs
- O 1 to 5 flights of stairs
- O 6 to 10 flights of stairs
- O More than 10 flights of stairs

42. If you went somewhere in your hometown, what kind of transportation did you use?

- O I never go out
- O Car
- O Public transportation
- O Bicycle
- O Walking

43. How often do you go out for shopping?

- O Never or less than once a week (Go to question 45)
- O Once a week
- O Twice a week
- O Every day

44. If you went out for shopping, what kind of transportation did you use?

- O Car
- O Public transportation
- O Bicycle
- O Walking

45. Did you play a sport last week?

O Yes
O No (Go to question 47)

46. Please indicate the type of sport and the number of hours you played last week.

Type of Sport	Hours per Week

47. Did you have other physically active activities last week?

O Yes
O No (Go to question 49)

48. Please indicate the type of activity and the number of hours.

Type of Activity	Hours per Week

49. Did you watch more than 30 minutes of television a day?

O Yes
O No (Go to question 51)

50. If yes, how many hours a day did you usually watch television? ☐☐ Hour(s)

Next, some questions on diet.

51. Are you on any special diet for health reasons, such as a low salt diet or a low sugar diet?

O Yes
O No

52. Have you ever tried to make dietary changes?

O Yes
O No (Go to question 55)

53. Which of the following changes have you tried to make?
Also, which changes DID you <u>make and maintain</u> for a period of 6 months or longer?
(Check ALL that apply)

	Asked to Make	Did Make
Eat less red meat	☐	☐
Cut down on fat intake	☐	☐
Cut down on calories	☐	☐
Cut down on cholesterol	☐	☐
Lose weight	☐	☐
Eat more fruits and vegetables	☐	☐
Eat fewer dairy products	☐	☐
Other (please describe below):	☐	☐

_____

_____

54. In general, how difficult was it for you to make the dietary changes you noted?

Very Easy	Easy	Not Easy or Difficult	Difficult	Very Difficult
O	O	O	O	O

55. How much do you agree with the following statements?
(Please fill in one circle for each item)

	Strongly Disagree	Disagree	Partially agree and disagree	Agree	Strongly Agree
a. What I eat is important to the way I feel.	○	○	○	○	○
b. What I eat is important to my health.	○	○	○	○	○
c. Breast cancer is a very important health issue.	○	○	○	○	○
d. Heart disease is a very important health issue.	○	○	○	○	○
e. Osteoporosis is a very important health issue.	○	○	○	○	○
f. Lung cancer is a very important health issue.	○	○	○	○	○

56. Have you seen a dietician or attended nutrition classes in the last 3 months?

○ Yes
○ No (Go to question 58)

57. If yes, why? _____

_____

_____

58. Do you have any food allergies?

○ Yes
○ No (Go to question 60)

59. If yes, please identify these foods. _____

_____

_____

60. **How much has your diet changed from when you were growing up?**

Not at all	Very little	Don't Know	Quite a bit	Very Much
○	○	○	○	○

61. **Aside from this study, were you planning to make any of the following changes in your eating habits in the next 6 months?** *(Check ALL that apply)*

- ☐ Cut down on calories
- ☐ Cut down on fats
- ☐ Eat more fiber
- ☐ Cut down on cholesterol
- ☐ Eat less red meat
- ☐ Eat more fruits and vegetables
- ☐ Lose weight
- ☐ None of these. (Go to question 63)

62. **How positive do you feel that you can stay focused on your dietary goals during the next 6 months?**

Not at all Confident	A little	Don't Know	Quite a bit	Very Confident
○	○	○	○	○

63. **How important do you feel other peoples' support is in helping you change your diet?**

Not at all Important	A little	Don't Know	Quite a bit	Very Important
○	○	○	○	○

64. **How much support would you get for eating more vegetables....**
(Please fill in one circle for each item)

	Never	Rarely	Sometimes	Quite Often	Always
a. **from people at work?** *(Skip this question if not employed)*	○	○	○	○	○
b. **from close friends?**	○	○	○	○	○
c. **from your spouse or family?**	○	○	○	○	○

65. How important are the following in deciding which foods you eat?
(Please fill in one circle for each item)

	Never Important	Rarely Important	Sometimes Important	Usually Important	Always Important
Convenience	○	○	○	○	○
Taste	○	○	○	○	○
Appearance	○	○	○	○	○
Smell	○	○	○	○	○
Cost	○	○	○	○	○
Health	○	○	○	○	○
Ethics eg. Animal Rights	○	○	○	○	○
Religion	○	○	○	○	○
Social Concerns	○	○	○	○	○

66. In a typical week, how often do you eat meals or snacks?
(Please fill in one circle for each item)

Days Per Week

	0 to 1	2 to 3	4 to 5	6 to 7
Breakfast	○	○	○	○
Lunch	○	○	○	○
Dinner	○	○	○	○
SNACKS:				
Morning	○	○	○	○
Afternoon	○	○	○	○
Evening	○	○	○	○

67. In a typical week, how often do you eat the following meals <u>with other people</u> (family, friends, co-workers)? *(Please fill in one circle for each item)*

	Days Per Week: 0 to 1	2 to 3	4 to 5	6 to 7
Breakfast	○	○	○	○
Lunch	○	○	○	○
Dinner	○	○	○	○
SNACKS:				
Morning	○	○	○	○
Afternoon	○	○	○	○
Evening	○	○	○	○

68. In a typical week, how often do you eat the following meals OUTSIDE of your home (cafeteria, fast foods, vending machines, restaurant, at someone else's home)?
(Please fill in one circle for each item)

	Days Per Week: 0 to 1	2 to 3	4 to 5	6 to 7
Breakfast	○	○	○	○
Lunch	○	○	○	○
Dinner	○	○	○	○
SNACKS:				
Morning	○	○	○	○
Afternoon	○	○	○	○
Evening	○	○	○	○

69. How much do you agree with the following statements?

	Strongly Disagree	Disagree	Partially agree/disagree	Agree	Strongly Agree
a. I am open to trying foods that I never tried before.	○	○	○	○	○
b. Low-fat foods can taste good.	○	○	○	○	○
c. I enjoy the taste of most vegetables.	○	○	○	○	○

70. How confident are you that you can make healthy food choices when you....

	Almost never Confident	Rarely	Sometimes	Quite Often	Almost always Confident
a. are anxious (or nervous)?	○	○	○	○	○
b. feel physically run down?	○	○	○	○	○
c. are depressed (or down)?	○	○	○	○	○
d. are angry (or irritable)?	○	○	○	○	○
e. are bored or have nothing to do?	○	○	○	○	○
f. are pressured by others to eat?	○	○	○	○	○
g. have experienced failure?	○	○	○	○	○
h. think others will be upset if you don't eat?	○	○	○	○	○
i. have to go out of your way to eat a healthy meal?	○	○	○	○	○
j. are ill or not feeling well?	○	○	○	○	○
k. are offered unhealthy but tasty foods?	○	○	○	○	○
l. are very hungry?	○	○	○	○	○
m. have limited time to plan your meal?	○	○	○	○	○
n. have many available unhealthy foods?	○	○	○	○	○
o. others offer you less healthy foods?	○	○	○	○	○
p. eat out (at restaurants, friends' homes, etc.)?	○	○	○	○	○
q. during holidays or special occasions?	○	○	○	○	○
r. are socializing with friends?	○	○	○	○	○

71. How do you feel about each of the following foods?
(Please fill in one circle for each item)

	I would never eat this food	I would eat this food if I had to	I sometimes like this food	I always like this food
Carrots	○	○	○	○
Cabbage	○	○	○	○
Broccoli	○	○	○	○
Asparagus	○	○	○	○
Brussels Sprouts	○	○	○	○
Green Peas	○	○	○	○
Radish	○	○	○	○
Kidney Beans	○	○	○	○
Cauliflower	○	○	○	○
Zucchini	○	○	○	○

72. Do you eat beef or chicken less than four times in a week?

○ Yes
○ No

73. Are there any foods you dislike so much that you would never eat them?

○ Yes
○ No (Go to question 75)

74. If yes, please identify these foods. _____

_____

_____

75. Compared to most of your friends, family, and co-workers, how do you rank your present diet according to amount of ... *(Please fill in one circle for each item)*

		Lower in Fat		Average in Fat		Higher in Fat
a.	Fat?	○	○	○	○	○
b.	Vegetables?	○	○	○	○	○

	Lower in Vegetables		Average in Vegetables		Higher in Vegetables

76. What is the recommended highest percent of fat in the diet? **(Check ONE only)**

○ 10% ○ 20% ○ 30% ○ 40% ○ 50%

77. I am more likely to get heart disease, cancer or another serious disease if I ...

	Strongly Disagree	Disagree	Partially agree/disagree	Agree	Strongly Agree
a. eat a lot of high-fat foods	○	○	○	○	○
b. eat a lot of vegetables	○	○	○	○	○
c. eat a lot of fiber/roughage	○	○	○	○	○

78. Choose the vegetable with the most nutritional value. **(Circle ONE answer only)**

○ Iceberg Lettuce ○ Broccoli ○ They are the same ○ Don't know

79. Choose the vegetable with the most nutritional value. **(Circle ONE answer only)**

○ Spinach ○ Zucchini ○ They are the same ○ Don't know

80. Choose the vegetable with the most nutritional value. **(Circle ONE answer only)**

○ Summer Squash ○ Cauliflower ○ They are the same ○ Don't know

81. According to government recommendations, how frequently should fruits or vegetables be eaten?

- O 0 to 1 servings per day
- O 2 to 3 servings per day
- O 3 to 4 servings per day
- O 5 to 6 servings per day
- O More than 6 servings per day

82. For each food listed below, choose the cooking method that is the healthiest.

	Fried	Steamed	Baked	Boiled	Raw
a. Carrots	O	O	O	O	O
b. Broccoli	O	O	O	O	O
c. Onions	O	O	O	O	O
d. Chicken	O	O	O	O	

83. Which vegetable do you eat more frequently? **(Circle ONE answer only)**

O Iceberg Lettuce O Broccoli O Same Amount O Don't know

84. Which vegetable do you eat more frequently? **(Circle ONE answer only)**

O Spinach O Zucchini O Same Amount O Don't know

85. Which vegetable do you eat more frequently? **(Circle ONE answer only)**

O Summer Squash O Cauliflower O Same Amount O Don't know

86. Typically, how frequently do you eat vegetables?

- O 0 to 1 servings per day
- O 2 to 3 servings per day
- O 3 to 4 servings per day
- O 5 to 6 servings per day
- O More than 6 servings per day

87. For each food listed, circle the cooking method you typically use.

	Fried	Steamed	Baked	Boiled	Raw
a. Carrots	O	O	O	O	O
b. Broccoli	O	O	O	O	O
c. Onions	O	O	O	O	O
d. Chicken	O	O	O	O	

88. We will be contacting you by telephone several times over the study period. What time(s) of the day are you most likely to be home?

Day of the Week	Good Times to Call	Bad Times to Call
Sunday		
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		

Thank you for taking the time to complete this questionnaire!

4320007758

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BRASSICA
Vegetable and Food Questionnaire

Date Form Completed
Month / Day / Year

First Initial	Middle Initial	Last name

Please tell us how often you have eaten the specified food item, and the typical portion size in the past seven days, excluding today. All portion sizes refer to cooked size unless otherwise noted. Please write in the number of times that you have consumed the food and check off your usual portion size as compared to the Comparison Portion Size.

For example, if you ate broccoli three times (one cup at one sitting and ¼ cup the other two times:

	Number of Times Eaten	Comparison Portion Size	Your Average: Half this Size	Equal to this Size	Twice this Size
eg. Broccoli	03	½ cup	☐	☐	☐

Food Item	Number of Times Eaten	Comparison Portion Size	*Your Average: Half this Size*	*Equal to this Size*	*Twice this Size*
Broccoli		½ cup	☐	☐	☐
Brussel Sprouts		4 sprouts	☐	☐	☐
Cabbage		½ cup	☐	☐	☐
Cauliflower		½ cup	☐	☐	☐
Chinese Cabbage		½ cup	☐	☐	☐
Collard Greens /Swiss Chard /Kohlrabi		½ cup	☐	☐	☐
Mustard Greens or Turnip Greens		½ cup	☐	☐	☐
Rutabaga /Turnips		½ cup	☐	☐	☐

Food Item	Number of Times Eaten	Comparison Portion Size	Your Average: Half this Size	Your Average: Equal to this Size	Your Average: Twice this Size
Kale	☐☐	½ cup	☐	☐	☐
Spinach	☐☐	4 sprouts	☐	☐	☐
Onions	☐☐	1 sm or ¼ cup	☐	☐	☐
Carrots	☐☐	1 med or ¼ cup	☐	☐	☐
Sweet Potatoes	☐☐	¾ cup	☐	☐	☐
Soybeans - whole	☐☐	1- 8 oz. Glass	☐	☐	☐
Soy milk 8oz. Glass	☐☐	¾ cup	☐	☐	☐
Tofu	☐☐	½ cup	☐	☐	☐
Tempeh	☐☐	½ cup	☐	☐	☐
Broccoli Sprouts	☐☐	½ sprouts	☐	☐	☐
Alfalfa /Clover /Mung Bean /Soy sprouts (raw)	☐☐	½ cup	☐	☐	☐
Pinto Beans /Round Split Pea Pods	☐☐	½ cup	☐	☐	☐
Fresh Green or Mung Beans	☐☐	½ cup	☐	☐	☐
Garbanzo, Kidney Beans, or Black-eyed, Yellow Split or Chinese Peas	☐☐	½ cup	☐	☐	☐
Peas	☐☐	½ cup	☐	☐	☐
Lentils /Dal	☐☐	½ cup	☐	☐	☐
Seaweeds eaten dry (e.g. dulse, purple laver, nori)	☐☐	¼ cup or 7" sq sheet	☐	☐	☐
Seaweeds eaten cooked or soaked (e.g. arame, kombu, kelp)	☐☐	1 Tbsp or 2" sq sheet	☐	☐	☐
Apples	☐☐	1 med or ½ cup	☐	☐	☐
Bananas	☐☐	1 medium	☐	☐	☐
Apricots	☐☐	2 medium	☐	☐	☐
Nectarines	☐☐	1 med or ½ cup	☐	☐	☐
Peaches	☐☐	1 med or ½ cup	☐	☐	☐

Food Item	Number of Times Eaten	Comparison Portion Size	Your Average: Half this Size	Your Average: Equal to this Size	Your Average: Twice this Size
Strawberries		½ cup	☐	☐	☐
Grapefruit		½ Grapefruit	☐	☐	☐
Lemon, squeezed		¼ medium	☐	☐	☐
Orange		1 medium	☐	☐	☐
100% Fruit Juice (any type)		1- 8 oz. Glass	☐	☐	☐
Other Soy products not listed above.		Your Portion Size: _____			

Please Specify: _____

7539051190

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BRASSICA
Dietary Reactions

Date Form Completed
Month / Day / Year

First Initial | Middle Initial | Last name

We are interested in the effects of this intervention. Have you experienced any of the following conditions in the last week

- ☐ dizziness
- ☐ nervousness
- ☐ irritability
- ☐ fatigue
- ☐ tremor
- ☐ diarrhea
- ☐ constipation
- ☐ loss of appetite
- ☐ blurry vision
- ☐ eye irritation
- ☐ weight gain
- ☐ weight loss
- ☐ dry skin
- ☐ yellowish skin
- ☐ loss of hair
- ☐ gas/flatulence
- ☐ difficulty remembering / difficulty thinking clearly
- ☐ muscle pain
- ☐ muscle weakness
- ☐ depression
- ☐ sleep disturbances (including insomnia)
- ☐ swelling

Please indicate any other conditions that you have experienced, *if any*.

_____

_____

○ I have not experienced any of the above reactions this week.

Appendix 3
Recruitment and Consent

OCTOBER IS....

Breast Cancer Awareness Month

Dear

Most women do not realize that once they've conquered breast cancer, there are still things they can do to help themselves. We, here at the University of South Carolina's Norman J. Arnold School of Public Health, are inviting you to take part in a study. It focuses on foods that show some promise for reducing harmful types of estrogen, thus possibly reducing risk of recurrence.

In the next few weeks we will be calling you to ask for your participation in this study. Your involvement would be greatly appreciated. Not only may you benefit but your participation may help other women at risk for this disease. Please remember, without your active support, there is no chance for a cure.

Thank you for your support!

UNIVERSITY of SOUTH CAROLINA

«Pt_First_Name» «Pt_Last_Name»
«Pt_Address_Line_1»
«Pt_City», «Pt_State» «Pt_Zip»

[Insert Header]

Dear Ms. «Pt_Last_Name»,

Breast cancer affects millions of people every day in South Carolina. It touches the lives of your friends, your family, and perhaps even you. Research has led to progress against breast cancer – better treatments and improved quality of life.

Your diet is an important part of your life, and the foods you eat may help to prevent disease. We are inviting women to participate in a short-term dietary study called the Brassica Health Study. This study will help to determine whether or not simply eating certain vegetables every day may reduce the risk of developing breast cancer.

We will be calling you in the next few days to answer any questions that you might have at this time. Once we are sure that all your questions are answered and you are willing to participate, we will mail you a brief questionnaire. If you do not wish to be called, please call my assistant, Vickie Smith at 777-7666 and leave a message that you do not wish to be called.

Your participation benefits everyone. You are helping to improve the health and quality of life of your children and grandchildren.

Thank you in advance for your help.

Yours Sincerely,

James R. Hebert, MSPH, ScD
Professor
Department of Epidemiology and Biostatistics
Norman J. Arnold School of Public Health
University of South Carolina

PALMETTO HEALTH

Review Date	Approval Begins	Approval Ends	IRB Number
July 3, 2002	July 3, 2002	July 2, 2003	#2000-78

DATE: July 3, 2002

TO: James R. Hebert, Sc.D.

FROM: Edward W. Catalano, M.D.
Chairman, Institutional Review Board (IRB)

SUBJECT: **Approval of Renewal: Protocol #2000-78 with Modifications**
35 subjects enrolled to date with an enrollment goal of 90.

On July 3, 2002, the convened Institutional Review Board approved your protocol and consent form entitled:

PHA IRB #2000-78: Phase I Induction and Estrogen Metabolism in Women with and without Breast Cancer.

Approval is effective from July 3, 2002 until July 2, 2003. Unless the IRB has waived the requirement for documentation of informed consent, attached is the IRB approved consent/assent document(s) to be used when enrolling subjects. [Note, this dated consent is to be used as a master to copy for all patient consents. Subjects can only be enrolled using consent/assent forms which have a valid "IRB Approval" stamp. A copy of the signed consent/assent form must be given to every study participant. A copy of the signed consent/assent form must be kept with your study records and in the patient's medical chart.] Prior to the end of this period, you will need to submit a copy of the institutional synopsis forms for renewal of the protocol, which must be completed and returned to the IRB at the Research Administration office, so that the protocol can be reviewed and approved for the next period.

In implementing the research activities, you are responsible for complying with IRB decisions, conditions and requirements. The research procedures should be implemented as approved in the IRB protocol. No changes in the protocol or consent may be made without prior IRB approval. Any significant deviations on proposed changes must be reported to the IRB. If your study uses an IND, all administered dosages are to be documented in the patients' chart. Storage and administration of the study drug are to be documented as being in accordance with FDA requirements.

Please refer to the Palmetto Health Alliance Investigator Brochure regarding investigator responsibilities with respect to the ethical use of human subjects in research. If you have questions or need additional information, please contact the Research Administration office at 434-4899. Please forward a copy of any publication(s) resulting from this research to the IRB, for their information.

DATA EXCLUSION VERIFICATION
LAPSE OF IRB APPROVAL

DATE: July 3, 2002

TO: James R. Hebert, Sc.D.

FROM: Edward W. Catalano, M.D.
Chairman, Institutional Review Board (IRB)

RE: **IRB Approval Lapse for IRB #2000-78**

The IRB approval for your application entitled,

PHA IRB #2000-78: Phase I Induction and Estrogen Metabolism in Women with and without Breast Cancer. (protocol title to be listed here)

expired from June 15, 2002 to July 2, 2002. As you are aware, no data may be collected, analyzed or reported for research purposes on studies without IRB approval. For our records, please sign in the space provided below indicating which of the two situations have taken place and **return to my attention by August 2, 2002.** If you have any questions or concerns, please contact the IRB Office at 434-4899. Thank you for your prompt attention to this matter.

All data collected and/or analyzed for research purposes on the above named application during said period of time **has been discarded** and will not be included in the study data for publication or presentation.

_____ _____
Principal Investigator's signature Date

OR

No data collection and/or analysis took place for research purposes on said application during the time period listed above.

_____ _____
Principal Investigator's signature Date

Note: This data exclusion verification form is not intended to restrict federally mandated reporting requirements on investigational drugs or devices to the FDA.

Palmetto Health Alliance

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

IRB#: 2000-78

TITLE: Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention.

PRINCIPAL INVESTIGATOR: James R. Hebert, Sc.D.

RESEARCH SUBJECT'S NAME: _____ **DATE:**_____

SPONSOR: United States Department of Defense

INVITATION TO TAKE PART AND INTRODUCTION:

You are invited to volunteer for a research study. You have been asked to be in this study because you have undergone a screening procedure at the South Carolina Cancer Center within the Palmetto Health Alliance (Columbia, S.C.) to see if you might have breast cancer .

PURPOSE OF THE RESEARCH:

The main purpose of this study is to determine if a 6-session dietary education program can help women incorporate into their diet certain foods that could alter levels of hormones thought to influence the risk of breast cancer. These foods are members of the *Brassica* genus. The most commonly consumed of these vegetables include cabbage, broccoli, cauliflower, and Brussels sprouts. The results of this study will help to develop dietary guidelines directed towards breast cancer prevention and altering the course of disease in women with breast cancer.

YOUR RIGHTS: It is important for you to know that:

- YOUR PARTICIPATION IS ENTIRELY VOLUNTARY.
- YOU MAY DECIDE NOT TO TAKE PART OR DECIDE TO QUIT THE STUDY AT any TIME.
- YOU WILL BE TOLD ABOUT ANY NEW INFORMATION OR CHANGES IN THE STUDY THAT MIGHT AFFECT YOUR PARTICIPATION.
- THE QUALITY OF CARE YOU RECEIVE AT THE HEALTH CENTER WILL NOT BE AFFECTED IN ANY WAY IF YOU DECIDE NOT TO PARTICIPATE OR IF YOU WITHDRAW FROM THE STUDY.

IRB APPROVAL

07-03-2002 - 07-02-2003

Subject's Initials _____ Witness's Initials_____

RANDOMIZATION:

Because it is not known whether changes in diet are effective in breast cancer prevention, not everyone in the study will be assigned to receive the dietary intervention. You will be assigned to one of two groups. One group will receive the dietary intervention, one group will not. This will make it possible for us to judge the effect of eating these vegetables in the fairest, most impartial way possible because the process of randomization ensures that the two groups of people (those receiving and those not receiving the intervention) are similar in other ways. The decision as to whether you receive the dietary intervention or not will be made by chance, like the flip of a coin, not by your doctor or based on your medical condition. You will have a 50% chance of receiving the intervention.

PROCEDURES:

This dietary study will last about two months per participant, and 90 women will participate. You will be asked to participate in a total of 12 different 24-hour dietary recalls during the study period (before, during, and after). If you are assigned to the dietary intervention, you will be asked to meet with a study dietitian for a one-hour individual session. This session will be followed by 6 two-hour group sessions over a three or four week period. Approximately fifteen people will attend each class, and classes will be scheduled on one or two weekday evenings. These sessions will include: 1. classroom presentations during which we will provide information about the vegetables – their chemical properties and their effects on health; 2. a group cooking experience in which you will be asked to learn about preparing the foods; and 3. a chance to eat what you have cooked with other women in the group.

You will be asked to add about four commonly known vegetables to your diet during the six weeks of the intervention. We will <u>not</u> be asking you to restrict your diet, or limit the other foods that you eat, in any way. The dietary intervention is not a weight loss program. You may eat anything that you wish to eat, but we ask that you also eat about two or three servings per day of the vegetables promoted in the intervention classes. These classes are designed to help you incorporate these vegetables into your normal meals.

We will ask to schedule two clinic visits with you. The first clinic visit will be scheduled at a time before the intervention starts, and the second visit will occur near the end of the intervention. During each of these clinic visits, a blood sample will be drawn in the usual way, by inserting a needle into a vein in your arm. About 4 teaspoons (20 milliliters) of blood will be collected, and this blood will be used to determine if there are any changes in levels of the hormones that are thought to be important in modifying breast cancer risk. We will measure your weight and the circumference of your hips and waist. We will provide you with a small urine collection container to collect a first-morning urine sample, and this urine sample can be brought to the clinic when you have your blood drawn. It is important that this urine sample be collected before you eat that day. This urine sample will be used to determine if there are changes in the levels of certain female hormones (estrogens) that are excreted from your body in your urine. Additionally, urine samples will be used to determine the levels of chemicals that naturally exist in the foods you will be asked to eat. We also will collect a small number of breast cells. This will be done by a procedure called a fine-needle aspiration, using a needle similar to that used for drawing blood. The amount of material removed by a fine-needle aspiration is always very small, less than a one-quarter of a thimble-full. This material will be used to determine levels of enzymes that are important in regulating levels of female hormones (estrogens).

In summary, each of the two clinic visits will include:

- Collection of a blood sample
- Delivery of a first-morning urine sample
- Collection of a small amount of breast material by fine-needle aspiration
- Measurement of your weight, waist, and hips

Finally, you will be asked to complete several questionnaires about your present health, diet, medication use, and the current level of depression and anxiety. These questionnaires will be completed near the time of your clinic visit, and will require about one hour.

After the end of the week of your last class, you will be advised that you may remove the intervention vegetables from your diet.

ALTERNATIVES:

You may choose not to take part in this study. If so, then you would not have to do any of the things listed above. This would in no way affect other aspects of your treatment or medical care.

RISKS AND INCONVENIENCES:

Drawing blood may hurt slightly, and you might have a bruise. Occasionally a person may become dizzy or faint when blood is drawn and there is a slight possibility of infection or temporary nerve damage. There may be pain associated with the fine-needle aspiration. This pain is usually short-lived (i.e., less than 12 hours), and well tolerated. Pain medication, for example Tylenol or Advil, can be taken to relieve this pain, and Tylenol capsules will be available at the time of the biopsy. Stronger pain medication may be prescribed if you think it is needed. There may be a small amount of bleeding which would present no health risk. There is a slight possibility of infection. Sterile techniques are used to avoid infection, but antibiotics can be used to treat an infection if this occurs. There is a very slight risk of temporary nerve damage, which should begin to heal within a few days. There should be no risk from answering any of the study questions, or in providing a urine sample.

Sometimes people find a question on a questionnaire sensitive or uncomfortable to answer. While there are reasons why the question is asked, you do not have to answer a particular question if you feel uncomfortable to do so. Please remember, all results will remain confidential. When we do the statistical analyses for the entire study we will not reveal your identity or the identity of anyone else in the study.

Adverse or allergic reactions to the foods promoted by the dietary intervention are rare. Occasionally, individuals have reported that consumption of the intervention foods leads to excess gas or diarrhea. We will ensure that you are in weekly contact with the project nutritionist and other research staff, and we will encourage you to call if you suspect any side effects. If any side effects occur, you may be advised to eat fewer of the vegetables.

Incorporation of a few additional foods to the diet may at times be an inconvenience when dining out or visiting people. There also may be inconvenience when planning or preparing meals for others in your home. The intervention class content and project staff will try to provide as much help as reasonably possible to overcome such inconveniences and to make these changes enjoyable. Through discussion and

conversation, other classmates also may be able to help with these issues.

COMPENSATION IN CASE OF INJURY:

All forms of medical diagnosis, treatment and research, whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop complications from participation in this study. In the event of any injury resulting directly from the research procedures, neither the study personnel, the University of South Carolina, nor the Palmetto Health Alliance have made any provision for the payment of any financial compensation to you or to provide any financial assistance for medical or other costs.

This study is being funded by the Department of Defense and conducted by the United States Army in conjunction with the University of South Carolina. Army regulations provide that, as a volunteer in a study conducted by the United States Army, you are authorized all necessary medical care for any injury or disease that is a direct result of your participation in the research. The Principal Investigator or his designee will assist you in obtaining appropriate medical treatment under this provision, if it is required. If you have any questions concerning your eligibility for Army-funded medical treatment you should discuss this issue thoroughly with the Principal Investigator or his designee before you enroll in this study. This is not a waiver or release of your legal rights.

BENEFITS:

This study may be of no direct benefit to you. However, we will make study results available to you when the final results are compiled and written. At the end of the study, you may request a summary of all of your own results with a brief description of what they mean. As results from the entire study are published, we will advise you and you may request them as well. Additionally, the knowledge gained from your participation in this research may help to better understand how to prevent or treat breast cancer.

COSTS:

There will be no direct cost to you for participating in the study. The analyses of questionnaires, blood, urine, aspiration material, and the dietary intervention classes will be provided free of charge.

If you are assigned to the dietary intervention, you will receive a supply of vegetables during each class that can be incorporated into the regular diet. This is done as a convenience to you, and the amount of vegetables supplied should be more than enough to meet the intervention objectives. However, such supplies are intended to be eaten by the study participant, and there will not be a sufficient quantity to share with others. In the event that you wish to share the provided vegetables with friends or family members, we would ask that you purchase additional vegetables.

REMOVAL FROM STUDY

You may be taken out of the research study if it appears that you are unable to: keep your appointments, provide blood, urine, two fine-needle aspiration samples, or do not provide answers on the questionnaires. If this occurs, you will be given a full explanation.

IRB APPROVAL

07-03-2002 - 07-02-2003

Subject's Initials _____ Witness's Initials_____

CONFIDENTIALITY:

Your research records will be confidential. In all records of the study you will be identified by a code number and your name will be known only to the researchers. Your name will not be used in any reports or publications of this study.

Because this study is funded by the United States Department of Defense it has a special set of requirements known as "Volunteer Registry Data Base Requirements". It is the policy of the U.S. Army Medical Research and Materiel Command, the entity that regulates this research, that data sheets are to be completed on all volunteers participating in research for entry into this Command's Volunteer Registry Data Base. The information to be entered into this confidential database includes your name, address, Social Security number, study name and dates. The intent of the database is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

SAMPLE DONATION:

During this study, you will be asked to provide two breast fine-needle aspiration samples, two blood samples, and two urine samples. These samples will be used for hormone analysis related to breast cancer research. They also may be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. No commercial value is anticipated at this point. Should your donated sample(s) lead to the development of a commercial product, the University of South Carolina will own it and may take action to patent and license the product. The University of South Carolina does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have. You will not receive any notice of future uses of your sample(s).

PATIENT PROTECTION:

Further information on the research to be performed, or on any risks, benefits or alternative treatments may be obtained from James R. Hebert at 803-777-7666. This study has been approved by the committee to protect human rights for the Palmetto Health Alliance. Information concerning your rights as a research subject can be obtained by contacting the Office of Corporate Counsel at (803) 296-2124.

IRB APPROVAL

07-03-2002-07-02-2003

Subject's Initials _____ Witness's Initials_____

Consent to Participate in the research project IRB #2000-78, entitled:

Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

Subject's name: (printed or typewritten)_____
P.I. Name: James R. Hebert, Sc.D.

"The purpose and procedures of this research project and the predictable discomfort, risks, and benefits that might result have been explained to me. I have been told that unforeseen events may occur. I have had an opportunity to discuss this with the investigator and all of my questions have been answered. I agree to participate as a volunteer in this research project. I understand that I may end my participation at any time. I understand that there is a possibility that the blood, tissue, or urine samples, which I am providing under this study, may also be used in other research studies and could potentially have some commercial applicability. I have been given a copy of this consent form."

Person Obtaining Consent: _____

Subject's signature:_____ **Date:**_____

Subject's permanent address: _____

_____

Witness signature: _____ **Date:**_____

_____ _____
Witness' name (printed or typewritten) **Relationship to subject**

IRB APPROVAL

07-03-2002 - 07-02-2003

Subject's Initials _____ Witness's Initials_____

PHONE SCRIPT

Hello, may I please speak to _____.
(If participant is not available, ask whether participant lives at this address and when would be a better time to call back.)

My name is _____. We are currently conducting a study on women to see the role of specific foods in reducing the risk of breast cancer. This study is a collaborative effort between the South Carolina Cancer Center, the Palmetto Health Alliance, and the Norman J. Arnold School of Public Health.

We were given permission by Palmetto Richland Memorial Hospital and the University of South Carolina to contact women who have received breast cancer screening or breast health care at Palmetto Richland Memorial Hospital.

Do you have a few minutes for me to tell you a little more about this study to see if you may be interested in taking part?

If no, (either thank the participant for her time or enquire when would be a better time to call her back. Also record in the verification table of the ACCESS database the appropriate response and time to call back)

If yes, then continue:

The findings of this study will be important because very little is known about what a woman can do to affect breast health. Lifestyle choices, especially around eating, may hold the key to reducing the risk of breast cancer. In this study, we are interested in whether eating certain types of vegetables every day may help prevent disease. In our previous work we have seen that diet may play an important role in reducing breast cancer risk ***{Be ready to answer a few questions here- on published study results & role of estrogen in BC}.***

Description of the study:

For intervention group say this:
If you agree to be in the study, we will be asking you to eat certain vegetables during the four weeks of the study. To help you, we will provide you with vegetables, recipe books, and cooking classes. In addition to eating these vegetables, you will be asked to schedule two appointments at our clinic during which we will collect a urine sample and a tissue sample from your breast.

Just to let you know, this procedure would be performed by a trained professional and is simple and less painful than having blood drawn, though we understand that you may have some concerns about how it works. **{Be ready to answer a few questions here:** Using a syringe, a small sample of your tissue will be extracted. As I said before, this is a

simple and less painful process than having blood drawn. The visit shouldn't take more than about an hour in total. } We will also be taking some measurements such as your height, weight, abdominal circumference and percent body fat. We estimate that the entire visit should take approximately one hour.

For non-intervention group say this:
If you agree to be in the study, you will be asked to schedule two appointments at our clinic during which we will collect a urine sample and a tissue sample from your breast. Just to let you know, this procedure is less involved and less painful than having blood drawn. We will also be taking some measurements like height, weight, abdominal circumference and percent body fat. We estimate that the entire visit should take approximately one hour.

Also, if you are interested we will provide you with cooking classes and a recipe book at the end of the study period.

For both groups, say this:
We will also be sending you a questionnaire that you can complete over the next week and return to us. The only way that we can learn more about the effect of lifestyle choices is with your participation. Would you be interested in helping us? **or** Does this seem like something that you would be interested in doing?

[If no, thank the participant for her time:
Thank you for your time and have a nice day.]

If yes, continue: Thank You! Do you have about 10 minutes to spare so that I can ask you a couple of questions? (open the ACCESS database and record the information in the survey form from now on)

If no then record the best time to call back at weekdays and weekend in the verification part of the ACCESS database.

ASK QUESTIONS FROM THE QUESTIONNAIRE.

We can schedule your first appointment at your convenience during the next week.

When will be the best time to schedule your appointment?
(RECORD APPOINTMENT ON THE SURVEY FORM OF THE ACCESS DATABASE and then consequently transfer at the end of the day on the **CALENDAR)**

The appointment will be in the same clinic where you will be coming for your work-up. A staff member will meet you in the lobby.

We will also be taking waist, hip, and abdominal body measurements so we would like to ask that you refrain from exercising or sitting in a sauna within 8 hours of your appointment. In order for the measurements to be accurate, we also need you to refrain from alcohol for 12 hours prior to your appointment. Before agreeing to take part in the study, you will be asked to sign a consent form during this visit. Do you have any questions at this time?

Well, we've reached the end of the interview. We will be mailing out the questionnaire to you in the next few days. When you receive it, please take the time to read the directions and fill it out as well as you can. If you have any questions, you can call the project coordinator, Mary Modayil, at 777-6217.

On behalf of the entire Breast Health Intervention Study research staff I would like to thank you for participating in this study. Have a nice afternoon.

I have several questions that I would like to ask you to see if you may qualify to take part in this study (it should take about 10 minutes). Is this a convenient time for you?

If not, when would be a better time to call you?

Personal Characteristics: (Yes/No Responses)

Y 1. Are you at least 45 years of age?

Y b) Are you completely past menopause or past the change of life? (i.e., *have you had no period in the past 12 months: if no period then okay to be in study*)

c) Have you had a hysterectomy? If so, did the hysterectomy include a complete ovarectomy (i.e., were both ovaries removed)? (Note: *If participant is not sure if she is post-menopausal, this is question is a requirement. If participant is unsure, ask her to ask her physician)*

Y 2. Do you plan to live in South Carolina for the next six months?

Diet: (Yes/No Responses)

1. Would you be willing to increase your vegetable intake over the study period: if yes, then okay to be in the study
2. Are you on any special diet for health reasons such as a low salt diet or a low sugar diet? (i.e., would you be willing to increase your vegetable intake over the study period: if yes, then okay to be in study)

N 3. Do you drink more than 3 alcoholic drinks in a day?

Medication Use: (Yes/No Responses)

1. Do you smoke cigarettes or use any other tobacco product? Document this as "yes" or "no".

N 2. Are you taking any thyroid medications? *(Note that the participant will know this because she will be taking a pill every day for this.)*

3. Are you currently taking any hormone replacement medications or exogenous estrogens? Document this as "yes" or "no".
4. Do you take any over-the-counter hormones (e.g. melatonin, DHEA) or herbal remedies? Document this as "yes" or "no".
5. Do you regularly use Tagamet or Pepcid AC for indigestion or heart-burn? Document this as "yes" or "no".
6. Are you on any medication for hypertension or diabetes? Document this as "yes" or "no".
7. Are you currently taking any other diet or nutritional supplement regularly? (i.e., over 3 times per week) Document this as "yes" or "no".

Y 8. Do you expect your medications to remain constant for the next 6 months?

Health History: (Yes/No Responses)

N	1. If you have ever had cancer or a malignancy, are you undergoing or have you had radiation or chemotherapy for it in the past year?
N	2. Have you had a double mastectomy? Note: The participant can be in the study if she has at least 1 breast without an implant.
	3. Have you ever been diagnosed with a liver disease, such as cirrhoses? Document this as "yes" or "no".
N	4. Within the past year, have you been admitted to a psychiatric hospital?

When the participant comes to the clinic, she will need to bring in all medications and supplements that she is taking so that we can record the names, frequency of use and whether or not she will regularly be taking these meds./supplements.

Appendix 4
Collection & Processing

URINE PROTOCOL

Collection:

The sample will be collected on the day of the clinical visit in a standard sterile collection cup. To prevent oxidation of labile products, 100 mg ascorbic acid will be added to each cup prior to the urine collection.

Supply

1. Urine Collection cup.
2. 100 mg of Ascorbic Acid.
3. Plastic bags.
4. Paper bags.

Urine Collection

- Label the container with the participants ID number, initials, and the date.
- Ask participant to provide a urine sample. If she is able to do so at that time, a urine collection cup, a plastic bag and a paper bag will be provided to her.
- Give instructions for urine collection. "Please fill to this line on cup" and mark on cup at 50 ml line.
- Escorted to the ladies room. If she is not able to pass urine she will be asked to drink a glass or two of water and she can collect the sample anytime during the course of the visit.

- Record date and time on the tracking form.
- Check container lid for tightness.
- Store sample in refrigerator or cooler (if at Baptist site) at 4 degrees C until the sample is processed. (Must be placed in a biohazard sample bag).

PROCESSING SAMPLE (can be completed up to 5 hrs after collection).

- Aliquot approximately 1.25 mLs into 4 orange-topped cryovials.
- Place the cryovials in a -70° C freezer for long-term storage.

1. **Clinical appointment Instructions**

- You should not exercise or take a sauna before 8 hours of the study.
- You should refrain from alcohol intake for 12 hours prior to the study.

ANTHROPOMETRIC MEASUREMENT PROTOCOL

This section contains the standardized procedures for the anthropometric measurements (height, weight, and the abdominal, waist, and hip circumferences).

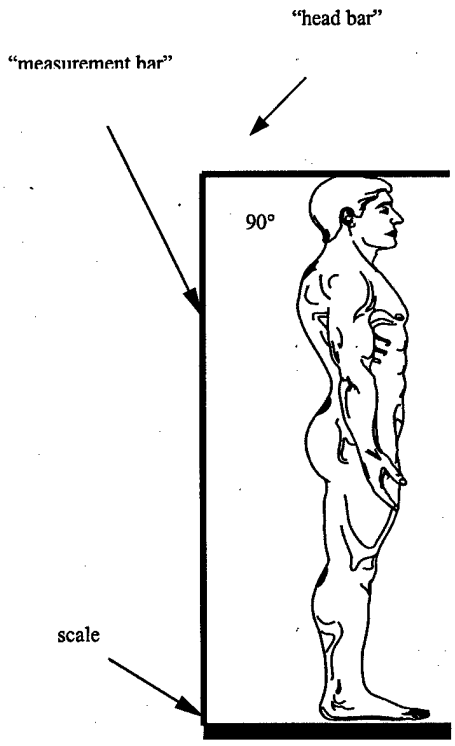
Instruments and Supply:

1. Scale for measuring height in feet and inches. (Qty.: 2)
2. Weighing scale with units in pounds (Qty.: 2)
3. Measuring tape (Gulick II tape measure) with units in centimeters. (Qty.: 2)

In order to approximate, as closely as possible, the participant's size and weight without clothes, all measures should be made **without shoes** and **without extra clothing**, such as sweaters, jackets, or coats.

Height

- Raise the height "measurement bar", extend the "head bar", and have participant step up on the scale facing out into the room
- They should be standing up straight, be looking directly ahead, and have her arms resting comfortably by their side
- To make the measurement, have the participant stand up straight, take a breath and exhale, and then, using two hands, lower the measurement bar down until the head bar rests on the crown of the head.
- The "head bar" should be at a 90° angle to the "measurement bar"
- Have the participant step down off the scale
- View the participant's height at the READ arrow on the



Body Weight

- Before taking individual measurements, be sure the scale(s) properly zeros when the "on" button is pushed. Also be sure that the scale is set to measure in pounds.
- To make a measurement, have the participant step up onto scale. Scale must read "0.0" before the participant steps onto the scale, otherwise the scale will register an error with a blinking display.
- **Record the weight <u>in pounds</u>, to the <u>nearest 0.1 pound</u>**

Circumference Measures

For all measures, be sure that the measuring tape is level (perpendicular to the ground), not twisted, and is pulled firmly around the participant.

Take all of the measures in order (waist, abdomen, and hip and record the values after each measurement. Repeat the measures at each site, again, in order. If two measures differ by **<u>more than 2 cm</u>**, repeat the measurement of that site a third and final time. Please record all measures taken (i.e., 2-3 per site).

To obtain measurements while holding a constant tension on the measuring tape, use the Gulick II tape measure. That is, when making a circumference reading, hold the cylinder end of the tape and pulling the tape tight across the participant. Be sure that the measurement is read from where the 0 mark at the beginning crosses the end of the tape.

Record the circumferences <u>in centimeters</u> (cm); to the <u>nearest 0.1 cm</u>. Participant's with circumferences greater than 150 cm (~ 60 inches) should be measured using a standard tape measure.

WAIST

- Face the participant; locate **the narrowest part of the torso** (or a site between the lower rib and the crest of the hipbone). You will have to poke and prod to find the correct site.
- Once the site is located, place the measuring tape around the torso and pull it snug. Check that the tape is level, and make the measurement.

ABDOMEN

- Have the participant point out her belly button. Lower the measuring tape to this spot, make sure the tape is level, and make the measurement **at the belly button**. If the location of the belly button is not at the greatest expansion of the participant's abdomen, take the measurement at that position.

HIP

- Have the participant turn to the side.
- Make the measurement at the largest expansion of the rear end, making the tape is level

Appendix 5
Intervention Materials

DIRECT Study: Class Schedule

Class 1

Goals

- Introductions
- Discussion: Describe major focus of study
- Explain study design, objective, and expectations

Events

- Introductions
- Distribute recipes and other written material
- Introduce study vegetables (snack time)
- Discussion: Overview of Brassica and Breast Cancer
- Diet Diaries

Take Home Messages

- Eat several servings a day of Brassica to reach study goals
- Do not changes other parts of the diet
- Record diet in diaries

Class 2

Goals

- Cooking practicum
- Discussion: Preparation of Brassica
- Summarize Diet Diary

Events

- Comments/Issues of Concern
- Describe active ingredient(s) in Brassica
- Cooking Practicum
- Preparation Techniques

Take Home Messages

- Continue to add Brassica to diet
- Record diet in diaries
- Plan for Pot-luck meal

Class 3

Goals

- Potluck dinner
- Discussion: Overall Health Effects of Brassica

Events

- Potluck dinner - B,K
- Discussion: Brassica and Health
- Distribute urine collection bottles and questionnaires

Take Home Messages

- Continue eating Brassica
- Focus on preparation methods
- Urine and blood collection week

Class 4

Goals

- Cooking practicum
- Guest speaker

Events

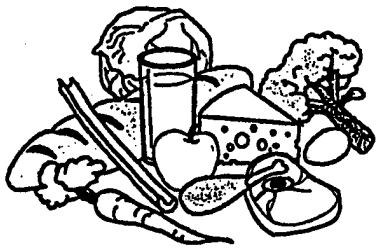
- Cooking practicum
- Guest speaker
- Study summary, discussion, and closing statements

Take Home Messages

- Continue with intervention diet, on your own, until blood drawn and urine collected
- Reminder that final urine/blood/24HR in about 3 weeks

DIRECT

Dietary Intervention to Reduce
the Risk of Breast Cancer



FOOD DIARY

Name: _____

Dates of Diary: _____, _____, _____

1. Record everything you eat or drink in your Food Diary for 3 days.
2. Look up the number of points on last page of diary for each Brassica serving, and record this number in the column labeled BRASSICA PTS.
3. Include comments about cooking and preparation for all Brassica foods.

Abbreviations: M/S = meal/snack, B=breakfast, L=lunch, S=snack, D=dinner

EXAMPLE Day: Wednesday, 4/1/98 (Brassica minimum goal = 10 points)

M/S	Amount	Foods and Beverages	Brassica pts	Preparation/Cooking
B	1	biscuit, 2" diameter	0	
	1 tsp.	jam	0	
	1 cup	coffee	0	
	3 tsp.	whole milk	0	
L	1	tuna sandwich	0	
	1 cup	broccoli	3	chopped, raw
	1 cup	cole slaw /white cabbage	3	chopped
	12 oz	Coca-Cola	0	
	1 oz	cheddar cheese	0	
S	1	Brussels sprouts	2	steamed
	8 oz	whole milk	0	
D	1 cup	beef stew, homemade	0	
	1 cup	tossed salad, (lettuce, tomato, onion, cucumber)	0	
	1/2 cup	savoy cabbage	3.5	chopped, raw
	1 cup	iced tea	0	
		Total Brassica	11.5	

1. Record everything you eat or drink in your Food Diary for 3 days.
2. Look up the number of points for every serving of Brassica, and record this number in the column labeled BRASSICA PTS.
3. Include comments about cooking and preparation for all Brassica foods.

Day 1: _____ (Brassica minimum goal = 10 points)

Abbreviations: M/S = meal/snack, B=breakfast, L=lunch, S=snack, D=dinner

M/S	Amount	Foods and Beverages	Brassica pts	Preparation/Cooking
		Total Brassica		

1. Record everything you eat or drink in your Food Diary for 3 days.
2. Look up the number of points for every serving of Brassica vegetable, and record this number in the column labeled BRASSICA.
3. Include comments about cooking and preparation for all Brassica foods.

Day 2: _____ (Brassica minimum goal = 10 points)

Abbreviations: M/S = meal/snack, B=breakfast, L=lunch, S=snack, D=dinner

M/S	Amount	Foods and Beverages	Brassica pts	Preparation/Cooking
		Total Brassica		

1. Record everything you eat or drink in your Food Diary for 3 days.
2. Look up the number of points for every serving of Brassica vegetable, and record this number in the column labeled BRASSICA.
3. Include comments about cooking and preparation for all Brassica foods.

Day 3: _____ (Brassica minimum goal = 10 points)

Abbreviations: M/S = meal/snack, B=breakfast, L=lunch, S=snack, D=dinner

M/S	Amount	Foods and Beverages	Brassica pts	Preparation/Cooking
		Total Brassica		

1. Find the vegetable and serving size that best fits what you ate.
2. If you ate more or less Brassica in a serving than what is listed here, adjust and record the Brassica points accordingly. For example, ½ cup of chopped broccoli = 1.5 points. It is not necessary to be more precise.

Minimum Goal = 10 Points

Vegetable	Serving Size	POINTS / SERVING
Brussels Sprouts	1 sprout	2
Savoy Cabbage	1 cup chopped	7
Savoy Cabbage	1 cup shredded	5
Kale	1 cup	4
Red Cabbage	1 cup chopped	3
Red Cabbage	1 cup shredded	3
Broccoli	1 cup chopped	3
Broccoli	1 cup flowerets	3
Broccoli	1 floweret	0
Collards	1 cup chopped	1
White Cabbage	1 cup chopped	3
White Cabbage	1 cup shredded	2
Cauliflower	1 cup chopped	3
Cauliflower	1 cup flowerets	2
Turnip	1 cup-cubed	1
Turnip	1 large	2
Turnip	1 med	1
Turnip	1 small	1
Turnip	1 slice	0

Dietitian Script and Methodology

1. For all interviews, the interviewer introduces him/herself by name, the study with which he/she is affiliated, and that (s)he is going to conduct a 24-hour diet recall. (S)he also will ask if this is a good time to talk. At this point, the dietitian will enter data on age and gender into the Header section of the NDS program.

2. To initiate the recall process, the interviewer will state the following "I would like to know what foods you ate after midnight yesterday, which was (state the day). Please tell me everything you ate or drank, including meals, snacks, beverages, candy, and alcohol. Start with the first thing you ate or drank and progress through the rest of the day. Please indicate approximately what time you had the items, whether it was a snack or a meal, and where you were. I will be entering this information directly onto a computer, so please speak slowly. After you have completed the list, I will be asking you some detailed questions about the recall." The control of the interview is then passed to the subject so (s)he can report food intake. Once the subject has begun to recall food intake, we try to not interrupt his/her train of thought - portion sizes, preparation information, etc. will be gathered in the next step. Some attention and encouragement are appropriate, such as "OK" or "what next." If the subject has difficulty getting started, we ask the subject to recall what (s)he did yesterday, and wait for the subject to start listing the food eaten. We always allow ample time for contemplation. When the subject has completed the 24-hour recall, the interviewer reviews the QUICK LIST and checks for snacks and alcoholic/non-alcoholic beverages and any other forgotten main food items.

3. After the subject has finished providing a description of his/her food intake, we employ standardized probing techniques as directed by the NDS system to assure that the foods are completely described, including detailed food preparation, size of portions eaten, items added to foods, etc. In addition to these on-line instructions we are aware that omissions often occur around snack items and foods taken in situations that are not typically considered eating (for example, using milk to "wash down" bed time medications). When the recall is completed, the interviewer asks the subject to hold the line while she quickly, but carefully, scans/reviews the FOOD REVIEW section of the NDS system to make sure the entries look correct (e.g., accidentally logging in "24 cups" instead of "24 ounces" of a calorie-containing beverage can produce a major difference in calories!). The interviewer should do this verbally, as often the subject may remember additional foods or detect errors as the review is conducted.

4. The interviewer then gathers the information to complete the TRAILER of the program. (S)he then thanks the subject for her/his time and if the situation warrants (i.e., the study requires that the subject be interviewed again) tells her/him that (s)he will be calling them again.

Missed interview protocol

When a call that you have been assigned cannot be completed, we would like you to make a 2nd and, if necessary, a 3rd attempt to contact the subject. First, check the patient information to see if there is revised information on "best time to call". Next, make the follow-up attempt on the first available day that corresponds to type of call day (weekday or weekend day) that was missed. For example, if the missed call is on the weekend, simply try to interview the subject on the next weekend day. If you are not available on a make-up date, please notify the project coordinator who will reschedule the interview.

Keep in mind that study subjects agree to participate and are expected to cooperate to complete the interviews. The project coordinator should be notified of any problems with cooperation. The project coordinator will then contact the site coordinator and enter a note into the study database.

Appendix 6
Preliminary Data Analysis

Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

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Abstract

In both ecological and clinical studies we have observed potentially protective effects of vegetables in the Brassica genus (e.g., broccoli, cauliflower, Brussels sprouts) on risk of breast cancer. Compounds in these foods may modify estrogen metabolism by causing 17β- estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16α-hydroxyestrone (16HE), the higher-risk metabolite. The indole glucosinolates (IGSL), which are contained in high concentrations in Brassica vegetables, are converted in the body to aryl hydrocarbon receptor (AhR) agonists. The activated AhR induces a number of protein products that can shift E2 metabolism away from 16HE and towards 2HE. AhR activation also induces immune system factors such as interleukin-1β (IL-1β) and other proteins, such as plasminogen activator inhibitor-2 (PAI-2), a protease inhibitor that has been associated with inhibition of tumor invasiveness (metastasis).

In order to examine the relationships between AhR, CYP1B1, IL-1b, PAI-2, estrogen metabolism, and IGSL from Brassica intake in relation to breast cancer risk we conduct two sequential studies: a cross-sectional comparison and a diet intervention. Our plan is to recruit 90 postmenopausal women, of whom one third will have had breast cancer and to compare these women on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1b; and 2) levels of relevant estrogens, E2, 2HE, and 16HE. Blood and fasting morning urine samples are collected from each woman at recruitment and then following the intervention for the purposes of measuring the estrogens, AhR activation, and levels of PAI-2 and IL-1β. Adipose tissue for assay of CYP1B1 will be collected by fine needle aspiration at the time of recruitment and at post-intervention follow-up. Currently, we have recruited and obtained baseline samples from 44 women (average age = 61.2 years). The women are slightly heavier than average, with a mean body mass index of 28.6kg/m^2. One third have had breast cancer. The first diet intervention group began in April 2002. Blood, urine, and a fine needle aspirate of breast tissue have been collected from each woman pre and post intervention. All such samples will be analyzed at the trial's end. Statistical analyses will examine the effect of the indole carbinols by fitting the data as continuous, which takes into account varying levels of compliance. Current analysis presents nutrient data collected using 24 hour dietary recalls.

Background

In a previous study of 46 very different countries:

- dietary factors accounted for most of the variation in breast cancer mortality
- on a per-calorie basis, high use of cabbage was associated with lowest mortality rates

Indole glucosinolates:

- high concentrations found in Brassica vegetables
- may induce aryl hydrocarbon receptor (AhR) and its protein products
- alter estrogen metabolism:

Aims

1. To determine if women with and without a history of previous breast cancer have different levels (in blood, urine and/or breast tissue) of:
   - aryl hydrocarbon receptor
   - its protein products (including CYP1B1, PAI-2, and IL-1β)
   - estrogen metabolites
2. To determine if intensive behavioral intervention on Brassica vegetable intake alters levels of CYP1B1 & estrogen metabolites.
3. To relate changes in CYP1B1 to levels of estrogen metabolites, both cross-sectionally and longitudinally.
4. To validate a self-administered two-page vegetable & fruit intake survey (VFQ) against a 24 hr dietary recall conducted by a nutritionist over the telephone.

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Design

- Cases: 45 postmenopausal women with previous breast cancer, any stage
- Controls: 45 women without a history of breast cancer
- Recruited from the Columbia, SC area, April 2002 – Dec. 2002
- African-American and European-American; matched on age only
- Randomized to Dietary Intervention or Non-Intervention groups

Pre-Intervention	During Intervention	Post-Intervention
24 hr diet recall	24 hr diet recall	24 hr diet recall
blood & urine		blood & urine
breast FNA*		breast FNA*
VFQ*	VFQ	VFQ

*(*FNA: fine needle aspiration; VFQ: vegetable and fruit questionnaire)*

Dietary Intervention

Three-weeks of nutritional classes (3 per wk):

•Taught by Registered Dietitian:

•Designed for general health (increase fruits, vegetables, whole grains, legumes; decrease fats, sugars)

•Includes recipes, cooking classes, how to read food labels

•Brassica vegetables, enough for one week, are provided to each participant.

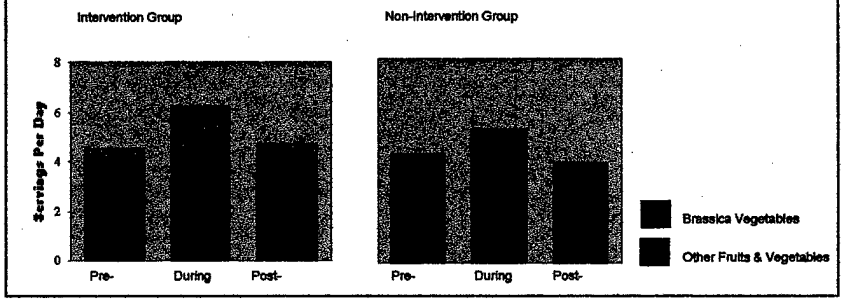
•Telephone coaching during the intervention, by the dietitian.

General Characteristics by Intervention Status

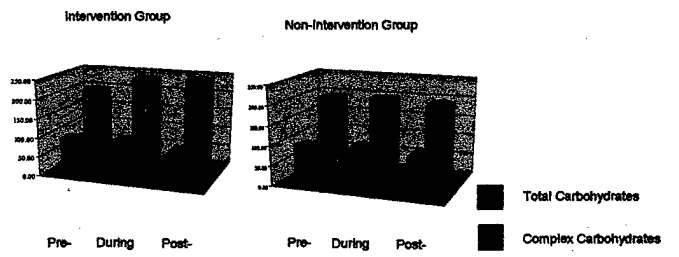
Variable	Non-Intervention	Intervention	Test of Difference*
Age (years)	62.7 (10.0)	59.4 (8.5)	p=.23
Education			
Some college or less	15	12	p=.58
Bachelor Degree or more	8	9	
Marital Status			
Married/Living with Partner	15	14	p=.92
Not Married	8	7	
Ethnicity			
White	19	14	p=.23
African-American	4	7	
Employment Status			
None	11	9	p=.67
Full-Time	6	4	
Part-Time	6	8	
History of Breast Cancer	4	3	p=.73
Body Mass Index (kg/m^2)	26.8 (6.1)	30.6 (8.4)	p=.10
Waist-to-Hip Ratio	0.80 (.05)	0.78 (.06)	p=.17

*Based on the t-test of differences across groups or the Chi-Square test of heterogeneity across levels of the variable of interest

Vegetable and Fruit Intake



Carbohydrate Intake



Dietary differences by Intervention Status

Variable	Pre-Intervention Phase (n=44)					Intervention Phase (n=39)				
	Intervention group (n=21)		Control Group (n=23)		Test of Difference*	Intervention group		Control Group		Test of Difference*
	Mean	(SD)	Mean	(SD)		Mean	(SD)	Mean	(SD)	
Energy (kcal/d)	1801	(469)	1820	(416)	0.89	1835	(609)	1911	(288)	0.55
Percent Fat (%CF)	35.0	(6.2)	32.6	(7.4)	0.51	31.4	(6.7)	31.4	(7.4)	1.00
Total Carbohydrates (g/d)	168	(69)	166	(55)	0.99	237	(173)	195	(40)	0.31
Complex Carbohydrates (g/d)	93	(31)	103	(32)	0.28	101	(66)	98	(27)	0.77
Dietary Fiber (g/d)	15.1	(6.0)	17.1	(6.1)	0.41	19.0	(8.3)	16.3	(5.6)	0.28
β-Carotene (ug/d)	3995	(1971)	5408	(4232)	0.16	6852	(3160)	4492	(2741)	0.21
Vitamin E* (IU/d)	69.0	(62.1)	73.4	(60.6)	0.69	73.6	(109.1)	60.2	(113.0)	0.65
Vitamin C* (g/d)	164	(161)	134	(74)	0.20	486	(1059)	126	(69)	0.17
Folate* (mg/d)	693	(363)	539	(388)	0.19	702	(377)	501	(314)	0.37
Iron (mg/d)	16.5	(8.1)	16.7	(8.6)	0.40	16.1	(8.1)	16.6	(7.6)	0.68
Total Fruits & Vegetables (srv/d)	4.5	(1.4)	5.2	(3.5)	0.35	6.2	(2.6)	4.3	(1.9)	0.01
Total Brassica (srv/d)	0.58	(0.58)	0.64	(1.05)	0.77	2.71	(1.57)	0.49	(0.66)	<.0001

*Based on dietary equivalence values calculated for these nutrients.

Conclusions

Results of the study obtained thus far show that the intervention is producing a large effect on consumption of Brassica vegetables. Some (about half) of the increase is displacing other fruits and vegetables. Changes in carotenoid, complex carbohydrate, and dietary fiber intakes are consistent with this pattern of increased Brassica vegetable intake. Total caloric intake is remaining about the same in the intervention group, indicating that the half not contributing to total fruit and vegetable intake is displacing other calorie-contributing foods – and is consistent with our counseling study participants in the intervention group that they eat an isocaloric diet. On all factors the control group is similar to the intervention group at baseline (though BMI is marginally higher). However, during the intervention period the control subjects decrease their intake of most foods – a phenomenon that we have observed in other "watched" populations. These dietary differences underline the importance of collecting such data for use as control variables in the analyses aimed at the primary study aims. By December of 2002 we will have finished all primary data collection for this study and will begin formal testing of study aims.